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Induced Biochemical Interactions in Immature and Biodegraded Heavy Crude Oils

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ABSTRACT

Studies in which selective chemical markers have been used to explore the mechanisms by which biocatalysts interact with heavy crude oils have shown that the biochemical reactions follow distinct trends. The term "biocatalyst" refers to a group of extremophilic microorganisms which, under the experimental conditions used, interact with heavy crude oils to (1) cause a redistribution of hydrocarbons, (2) cause chemical changes in oil fractions containing sulfur compounds and lower the sulfur content, (3) decrease organic nitrogen content, and (4) decrease the concentration of trace metals. Current data indicate that the overall effect is due to simultaneous reactions yielding products with relatively higher concentration of saturates and lower concentrations of aromatics and resins. The compositional changes depend on the microbial species and the chemistry of the crudes. Economic analysis of a potential technology based on the available data indicate that such a technology, used in a pre-refinery mode, may be cost efficient and promising. In the present paper, the background of oil biocatalysis and some recent results will be discussed.

Background

Biochemical reactions which occur during the interaction of a select group of biocatalysts associated with extremophilic microorganisms and crude oils follow distinct trends which can be monitored by means of characteristic chemical markers. The chemical markers represent several groups of compounds ranging from those containing sulfur and nitrogen to organometallic

compounds, saturates, aromatics, resins, and asphaltenes. Each group represents distinct chemical properties of oils which are affected by the action of biocatalysts. These markers are analogous to a group of compounds, known as "biomarkers" used in petroleum exploration, in source rock and reservoir correlations, as well as in maturation and degradation studies (Peters and Moldowan, 1993). Bioconversion of crude oils results in a reduction in sulfur content, a reduction in nitrogen content, and a reduction in the concentration of trace metals such as vanadium, nickel, arsenic, and others. Concurrently biochemical reactions lead to a redistribution of hydrocarbon fractions. The chemical markers serve as diagnostic tools by which several aspects of the biochemical conversion can be monitored (Premuzic et al., 1997; Lin et al., 1996, and Premuzic et al., 1994). Such monitoring defines the nature and the extent of bioconversion of the crude by biocatalysts, properties essential in the efficiency analyses of processes based on biochemical interactions with crude oils.

Typical examples of the action of several biocatalysts on immature and biodegraded heavy crude oils are given in Tables 1 and 2. Cerro Negro, CN, is a heavy Venezuelan oil. This oil is heavy because it has been biodegraded under reservoir conditions over geological time. During this process lighter hydrocarbons are lost in favor of increased concentration of high molecular weight factions. Therefore, it has been recommended that the term "bioconversion" (Premuzic et al., 1997) be used to refer to an oil which has been treated by a microbial biocatalyst under controlled experimental conditions (e.g., temperature, pressure, etc.) in an above ground reactor. OSC is a California offshore immature heavy crude, and MWS is Midway Sunset oil, chemically altered by steam. Comparable results for the action of different biocatalysts on the same heavy crude are given in Table 2. In this example the action of biocatalysts has been motivated by a single chemical marker. The oil used was Monterey, a

heavy biodegraded California oil. Changes in the hydrocarbon fractions due to bioconversion are characterized by chemical markers representing major oil fractions, e.g., saturates, aromatic, resins and asphaltenes. Examples of such changes for the three different heavy oils, an immature, a steam treated and a biodegraded are given in Table 3.

The utility of chemical markers in terms of the distribution of total heteroatoms and key hydrocarbon fractions is evident. The data also indicate variations in the efficiency of bioconversions. Extensive application of chemical markers has been used in the study of chemical and biochemical mechanisms by which biocatalysts induce the conversion of heavy crudes. Some of the recent results will be discussed in the following sections.

Experimental Procedures

Details of experimental procedures have been described elsewhere (Premuzic and Lin, 1991). Additional instrumentation used in the current studies will be mentioned briefly.

1. ICP-MS. Induced Coupled Plasma-Mass Spectrometer ICP-MS. A highly sensitive, ppt range, multi-element analytical instrument used for element analysis.
2. GC-MS, and Py-GC-MS: The Perkin Elmer 8700 gas chromatograph interfaced with ion trap mass spectrometer (GC-MS) and sulfur selective detector (FPD) for petroleum simulation distillation, analysis and composition analysis of saturates and aromatic hydrocarbons with selective detection for sulfur compounds. For heavy fractions a pyrolysis probe (Py-GC-MS) has been used which converts high molecular weight species into volatile fraction suitable for gas chromatographic analysis.
3. GC-MS^n Spectroscopy and PFPD. A Varian model Saturn 2000 gas chromatograph-ion trap tandem mass spectrometer (GC-MS^n). The instrument capable to operate in Electron Impact and Chemical Ionization modes with tandem mass spectrometric analyses of trace species of

mixtures containing same mass to charge (m/z) ratios. Concurrently, the GC effluent is split, so that a portion reaches a pulsed flame photometric detector (PFPD) for sensitive and specific analysis of sulfur, nitrogen, vanadium, nickel and other elements bound in organometallic compounds present in oils.

4. LC/MS^n. Finnigan model LCQ liquid chromatograph interfaced with a tandem mass spectrometer makes it possible to study separate polar fractions and their components by using liquid chromatography followed by mass spectrometric analysis of masses up to 4000 m/z. This instrument measures changes in the oil polar fractions as well as those occurring in the aqueous phase of a reaction mixture.
5. Infra-red (IR) and Ultraviolet-visible Spectroscopy (UV-VIS) is used as a routine basis to study reaction rates of biochemically induced changes in selected properties for example, aromaticity, optical density etc., which occur in the reaction mixtures. The instruments used are a Hewlett-Packard Model 8452A UV-VIS and a Perkin-Elmer dispersive Model 283IR spectrometer.

Results and Discussion

The oils used in experimental studies were heavy with °API gravities ranging from 12 to 20. Low °API (<20) gravity oils are low in gasoline and high in residuum, which means that as the oils become heavier, the H/C ratios decrease as the oils change from light to heavy, while the NSO/C ratios increase and the oils become richer in asphaltenes (Hunt 1979). Thus, the heavier fractions of oils are richer in resins, asphaltenes, and polar compounds containing heteroatoms (O,N,S) and metals. Chemically, this also means changes in the concentration and distribution of paraffinic, naphthenic, and aromatic compounds with a progressive increase in the concentration of polyaromatic and heterocyclic compounds. The highest concentration of the heterocyclic

compounds is found in resins and asphaltenes. Based on the literature data (Dolbear et al., 1986), sulfur is primarily distributed equally between resins and asphaltenes. While nickel and vanadium are similarly distributed, the amount of metals appears to be higher in the asphaltene fraction. Nitrogen follows a similar pattern of distribution. Further, the condensed polyaromatic chemical structures contain free radical sites with highly reactive unpaired electrons. These sites are involved in complexation of metals as well as inter- and intra- molecular reactions and molecular rearrangements, as well as in hydrogen bonding and in the formation of charge transfer complexes. The combined effects of the mentioned functionalities, i.e. those due to reactions such as complexing at O,N,S sites and those due to unpaired electron rearrangements, such as redox reactions, also affect viscosity and micellar structures. It is these properties that also influence the biochemistry associated with the interactions of microorganisms and crude oils. Mechanistically, biocatalysts can be considered as reactive species entering colloidal, micellar and molecular solutions which react with active sites such as the heteroatoms, initiating a dispersion of micellar organization leading to a "depolymerization" of the heavy crude polymer as shown in Figure 1, where the dark dots represent heteroatoms and other reactive sites. Such multiple reactions are analogous to the well studied mechanisms of microbial interactions with particulate matter, mineral surfaces, membranes, and other chemical surfaces involving active sites (Weinberg, 1977; Ehrlich and Holmes, 1986; Atlas, 1984). The chemical markers discussed in this paper represent both the extent of the heaviness of the crude and the degree of biochemical conversion of the crude. Although these oils are complex mixtures representing different types of oils they all fall into definable categories. Chemically and biochemically facilitated changes in these categories involve multiple and simultaneous reactions within a complex matrix, which can be followed by chemical markers. Thus, gas chromatography-mass spectrometry analysis

(GC-MS) (Figure 2) of the biodegraded M851, the immature OSC and steam treated MWS with the biocatalyst follow a similar trend with varying degrees of efficiency, consistent with fractionation data given in Table 3. In the gas chromatographic analysis shown in Figure 2, the hydrocarbon distribution increases from lighter hydrocarbon to heavier molecular weight hydrocarbons requiring longer retention times. The overall trend is an increase in saturates and a decrease in aromatics. Corresponding sulfur and nitrogen specific gas chromatographic analyses are shown in Figure 3. The data shown in this figure are consistent and show qualitative and quantitative changes in the distribution of nitrogen and sulfur containing hydrocarbons after the treatment with the same biocatalyst (BNL-4-23). All the data shown in Figures 2 and 3 have been generated under identical experiment conditions, enabling a direct comparison of chromatograms for each set of chemical markers. Typical mass balance data for MWS and OSC are shown in Table 4. The data discussed thus far are also consistent with the proposed mechanisms of biocatalytic conversion of heavy crudes. Studies with model compounds have shown that several bacterial species are capable of degrading dibenzothiophene (DBT) (Krawiec, 1990). These species are considered as potential candidates for the biological desulfurization of petroleum. Desulfurization of DBT is thought to occur via two major pathways. The first is a sulfur-specific pathway (Isbister and Kobylinski, 1985; Kilbane, 1993; Izumi et al., 1994) in which the sulfur in DBT is oxidized and carbon-sulfur bonds are broken to release the sulfur. A second pathway is the Kodama pathway (Kodama, et al., 1970; Yamada et al., 1970), where desulfurization of DBT proceeds by an initial breakage of aromatic rings followed by the removal of sulfur. However, these strains are not effective in desulfurization of substituted benzothiophenes and other sulfur containing organic compounds in the crudes. While studies dealing with the "biodegradation" of single model compounds are a good first step in studying

the desulfurization of oils, they are not sufficient, because oil is a complex system containing many structurally different sulfur-containing organic compounds. Also, the mechanism postulated for a pure culture with a single compound may not be functional in a complex system where concurrent intramolecular and intermolecular reactions, involving nitrogen, oxygen and trace metals, may be operating. Further, reactions involving free radical mechanisms associated with asphaltenes, chemically important components of heavy crudes, should also be considered.

Biocatalysts capable of sulfur removal from heavy crudes must be active within commercially definable limits. BNL's aim is to optimize the selectivity and cost-efficiency of heteroatom removal. For example, mechanisms which facilitate sulfur removal from sulfur containing compounds in oil by pathways leaving the hydrocarbon moieties in the oil intact, thus retaining the fuel calorific value, are of primary importance.

Nitrogen in petroleum is classified as basic and non-basic. Basic nitrogen compounds with a relatively low molecular weight can be extracted with dilute mineral acids. The non-basic nitrogen compounds that cannot be extracted with dilute acids are generally the carbazoles, indoles, pyrroles, and porphyrins. We have reported denitrogenation of petroleum by several strains of bacteria in various oil samples (Premuzic et al., 1997). The mechanisms by which denitrogenation occurs have not as yet been elucidated.

The presence of metals (e.g., nickel, vanadium, arsenic, etc.) in petroleum increases gas and coke formation during catalytic cracking and results in reduced yields of gasoline. In addition, metals present in petroleum can cause corrosion damage in the refractory furnace and contribute to particulate emissions. The BNL process results in significant demetallization of crude oil. As with other chemical markers (vide infra), the extent of metal removal depends on the oil used and the bacterial strain (Premuzic et al., 1997).

Although, the various biochemical transformations involved in the upgrading of crude oil discussed above were treated separately, these transformations are linked processes, with some biotransformations occurring simultaneously while others maybe occurring sequentially. Therefore, the optimization strategies must consider concurrently all of the processes. Economic analyses of conceptual technologies based on biochemical upgrading of petroleum (BUP) using chemical markers has been discussed elsewhere (Premuzic et al., 1997; 1995; Lin, 1996).

Conclusions

- The use of multiple chemical markers makes possible to follow and study the biochemical conversion of heavy crude oils.
- Current data indicate that the bioconversion of crudes follows distinct trends characterized by redistribution of hydrocarbons, e.g., saturates and aromatics, with concurrent removal from the organic phase (oil) of heteroatoms and trace metals.
- The extent of biochemical conversion depends on the type of biocatalyst used and on the chemical nature of the oil being treated.

Acknowledgments

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1. Peters, K.E. and J.M. Moldovan. The Biomarkers Guide, Prentice Hall, 363 p., 1993.

2. Premuzic, E.T., M.S. Lin, H. Lian, W.M. Zhou, J. Yablon. The Use of Chemical Markers in the Evaluation of Crude Oil Bioconversion Products, Technology and Economic Analysis, Fuel Processing Technology, 52, pp. 207-233, 1997.

3. Lin, M.S., E.T. Premuzic, H. Lian, W.M. Zhou, and J. Yablon. Environmental Significance of Biocatalytic Conversion of Low Grade Oils, Third Annual International Petroleum Environmental Conference, Albuquergue, NM, 1996.

4. Premuzic, E.T., M.S. Lin, and B. Manowitz. The Significance of Chemical Markers in the Bioprocessing of Fuels, Fuel Processing Technol. 40, p. 227, 1994.

5. Hunt, J.M. Petroleum Geochemistry and Geology, Ch. 3, Freeman, San Francisco, 1979.

6. Weinberg, E.D. Microorganisms and Metals, Marcel Dekker, New York, p. 492, 1977.

7. Ehrlich, H.L. and D. Holmes (Eds.). Workshop on Biotechnology for the Mining, Metal Refining and Fossil Fuel Processing Industries, Wiley, New York, 1986.

8. Atlas, R.M. Petroleum Microbiology, MacMillan, New York, 1984.

9. Kraweic, S. Bacterial desulfurization of thiophenes: screening techniques and some speculations regarding the biochemical and genetic bases, Develop. Industr., Microbiol. 31, pp. 103-114, 1990.

10. Isbister, J.D. and E.A. Kobylinski, Microbial Desulfurization of Coal. In: Processing and Utilization of High Sulfur Coals, Coal Science and Technology Series, Elsevier, Amsterdam, No. 9, (Y.A. Attia, Ed.), pp. 627-641, 1985.

11. Kilbane, J.J. U.S. Patent 5,198,341, March 1993.

12. Izumi, Y., T. Ohshiro, H. Ogino, Y. Hine, and M. Shimao. Selective Desulfurization of Dibenzothiophene by Rhodococcus erythropolis D-1, Appl. Environ. Microbial. 60, pp. 223-226, 1994.

13. Kodama, K., S. Nakatani, K. Umehara, K. Shimizu, Y. Minoda, and K. Yamada. Microbial Conversion of Petro-sulfur Compounds, III. Isolation and Identification of Products from Benzothiophene, Agric. Biol. Chem. 34, pp. 1320-1324, 1970.

14. Yamada, K., Y. Minoda, K. Kodama, S. Nakatani, and T. Akasaki. Microbial Conversion of Petro-sulfur Compounds, I. Isolation and Identification of Dibenzothiophene-utilizing Bacteria, Agric. Biol. Chem. 32, pp. 840-845, 1970.

15. Premuzic, E.T., M.S. Lin, and H. Lian. Bioconversion of Heavy Crude Oils: A Basis for New Technology, Proceedings, The Fifth International Conference on Microbial Enhanced Oil Recovery and Related Biotechnology for Solving Environmental Problems, Eds. R. Bryand and K.L. Sublette, pp. 235-242, CONF-9509173 (DE-96001220), 1995.

16. Dolbear, G.E., A. Tang, and E.L. Moorehead. Upgrading Studies with Californian, Mexican and Middle Eastern Oils, Ch. 14, pp. 220-232, in ACS Symposium Series 34, Metal Complexes in Fossil Fuels, Eds. R.H. Filby and J.F. Branthaner, ACS 191st Meeting, NYC, 1986.

17. Premuzic, E.T. and M. Lin. Prospects for Thermophilic Microorganisms in Microbial Enhanced Oil Recovery (MEOR), Proceedings, SPE International Symposium, pp. 143-148, Anaheim, 1991.

Table 1. Effects of a Single Biocatalyst on Sulfur, Nitrogen, Nickel and Vanadium Concentrations

Oil	Biocatalyst		Chemical Marker % Reduction		
		S	N	Ni	V
CN	BNL-4-23	25	-	35	58
OSC	BNL-4-23	45	45	20	16
MWS	BNL-4-23	50	5	25	36

Table 2. Total Organic Sulfur Content and % Sulfur Removed After Bioconversion

Oil	Total %	% S Removed
UTM-851	1.84	
BNL-NZ-3	1.29	30
BNL-TH-31	1.56	15
BNL-TH-29	1.54	15
BNL-4-21	1.63	16
BNL-4-22	1.47	20
BNL-3-23	1.19	30
BNL-4-23	1.20	30

Table 3. Distribution of Four Major Fractions in Crudes Before and After Bioconversion with Biocatalyst BNL-4-23

	OCS		MWS		M-851	
Fraction	untreated	treated	untreated	treated	untreated	treated
saturate%	17.3	57.6	19.2	66.3	19.19	34.42
aromatic%	39.1	18.0	44.9	11.2	45.15	29.72
resin%	37.4	22.3	35.3	19.3	31.23	32.71
asphaltene%	6.20	5.65	2.60	3.18	4.44	3.57

Table 4. Biochemical Treatment of Midway Sunset Oil (MWS) and Offshore California (OSC) at 55°

Table 4

Biotreatment	%Oil recovered	Elemental Analysis			
		C	H	N	S
Control MWS untreated		86.45	10.99	0.79	1.00
MWS treated 4-23	110	86.02	11.67	0.64	0.50
Control OSC untreated		82.31	11.17	0.66	4.40
OSC treateed with 4-23	99	84.45	12.39	0.36	2.40

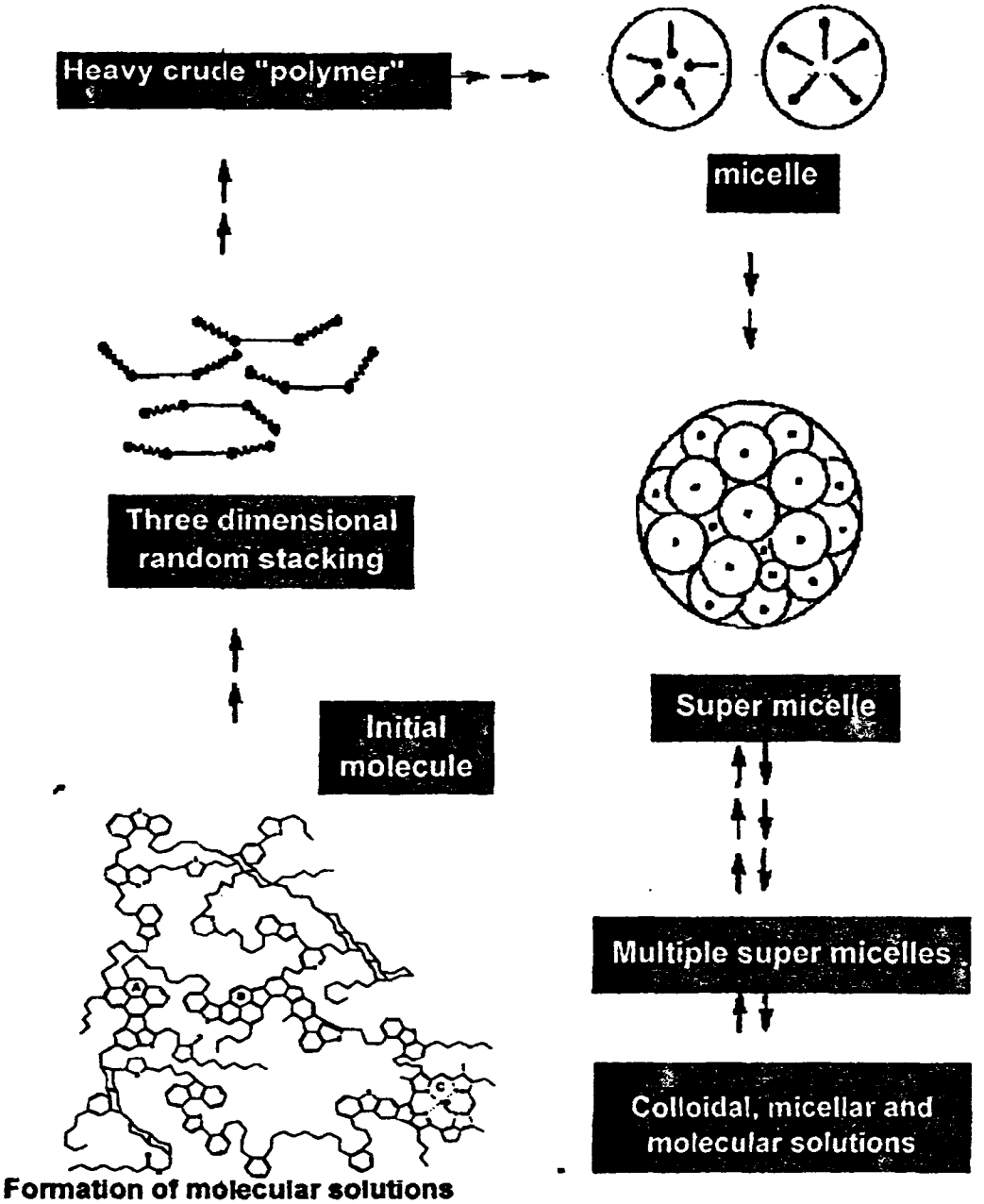

Figure 1. Involvement of Heavy Oil Fractions in the Evolution of a Fluid Oil Based (after Hunt and Yen). Dark Circles Represent Heteroatoms and Active Sites

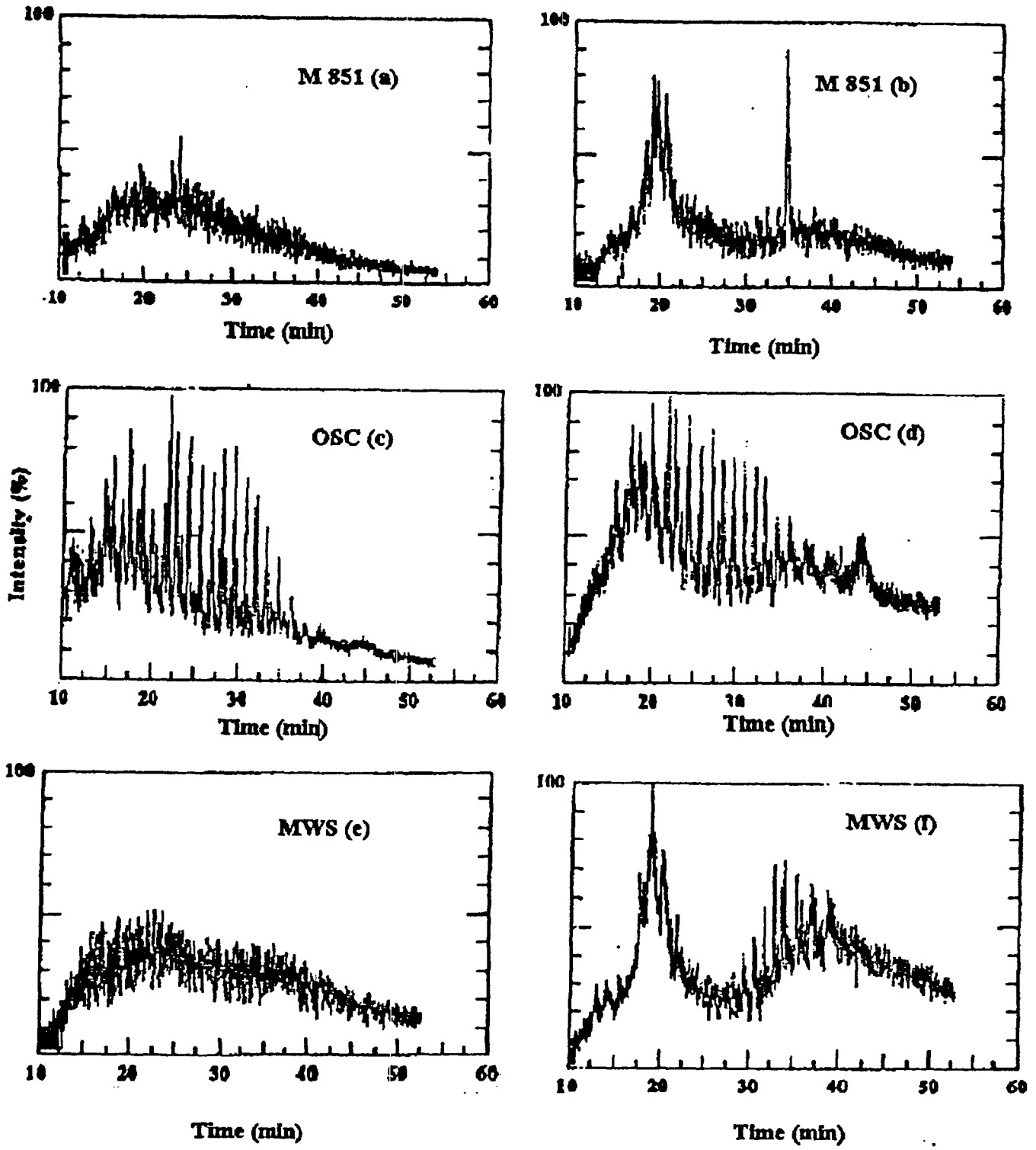

Figure 2. GC-MS Analysis M/e 57 Gas Chromatogram Traces of (a) M851 Control; (b) Treated with BNL-4-23; (c) OSC Control; (d) OSC Treated with BNL-4-23; (e) MWS Control, and (f) MWS Treated with BNL-4-23.

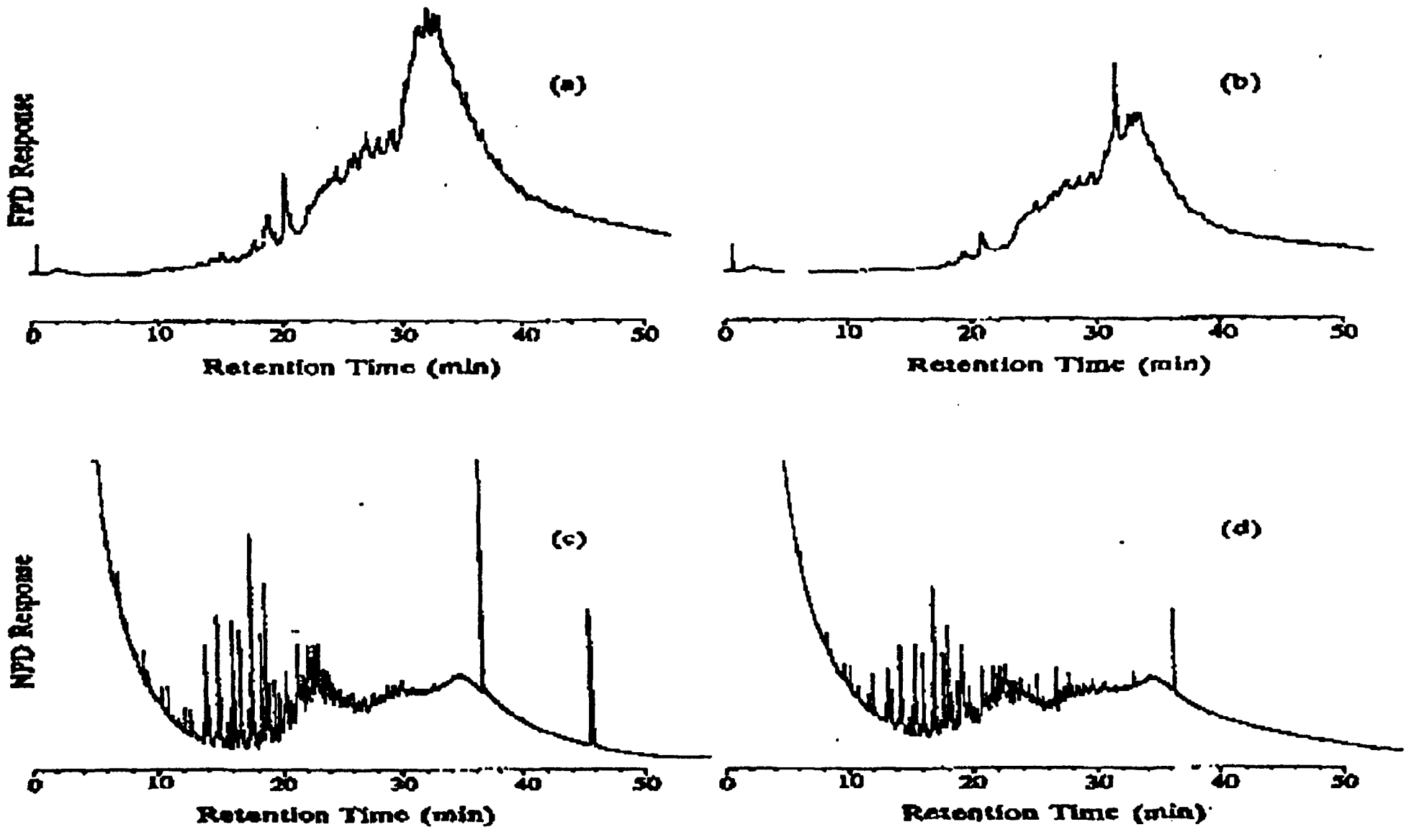

Figure 3. Specific Detector GC Analysis of OSC, Sulfur Specific Trace (Flame Photometric Detector (FPD)) (a) control; (b) OSC treated with BNL-4-23. Nitrogen Specific Trace (Nitrogen, Phosphorous Detector (NPD)); (c) Control; and (d) Treated with BNL-4-23.